SPECTROPHOTOMETRIC DETERMINATION OF NIMESULIDE, TRIBULUS TERRESTRIS, AND AMOXICILLIN IN AN ALKALINE MEDIUM, IN CLINICAL AND COMMERCIAL SAMPLES[**]

K. F. D. Pino, L. N. Oliveira, M. J. Silva, O. Caon-Filho, T. R. L. Dadamos[*]

Fundação Educacional de Fernandópolis, Fernandópolis, Av. Teotônio Viléla, Campus Universitário, SP, 15600-000, Brazil; e-mail: tonyrlrl@yahoo.com.br

This study focuses on the determination of drugs, namely nimesulide, amoxicillin, and tribulus terrestris, in an alkaline medium as well as in blood samples, urine, and commercial tablets. The study examines such factors as reproducibility, repeatability, robustness, and accuracy in the determination of the drugs in the samples.

Keywords: *drugs, determination, spectrophotometry.*

СПЕКТРОФОТОМЕТРИЧЕСКОЕ ОПРЕДЕЛЕНИЕ НИМЕСУЛИДА, ТРИБУЛУСА ТЕРРЕСТРИСА И АМОКСИЦИЛЛИНА В ЩЕЛОЧНОЙ СРЕДЕ В КЛИНИЧЕСКИХ И КОММЕРЧЕСКИХ ОБРАЗЦАХ

K. F. D. Pino, L. N. Oliveira, M. J. Silva, O. Caon-Filho, T. R. L. Dadamos[*]

УДК 543.42.062:615.03

Образовательный фонд Фернандополиса, Фернандополис, 1500000, Бразилия; e-mail: tonyrlrl@yahoo.com.br

(Поступила 25 августа 2017)

Исследование посвящено определению лекарств в щелочной среде и включает в себя идентификацию нимесулида, амоксициллина и трибулуса террестриса в щелочной среде (образцах крови и урины), а также в таблетках. Изучены такие факторы определения лекарств в образцах, как воспроизводимость, повторяемость, надежность и точность.

Ключевые слова: *лекарство, определение, спектрофотометрия.*

Introduction. Every process at some point undergoes analysis in order to ensure more reliable results. The analytical results are responsible for determining the decisions required for the quality control of products and the production process as well as developing new products and addressing environmental and health issues. Spectrophotometric methods are among the key applications of analytical chemistry, owing to their extensive use in clinical analysis laboratories. Molecular absorption spectroscopy forms the basis of many procedures to identify the functional groups of molecules, including drugs. Most of organic molecules and their functional groups are transparent in the region of the electromagnetic spectrum [1]. However, with chemical treatment it is possible to perform quantitative determination of the compounds that contain absorbent groups such as drugs [2].

Analytical methods are those in which the biological matrix is not used in the analysis, unlike the case of bioanalytical methods that are carried out in the biological matrix [3]. The choice of an appropriate analytical method is very important to ensure quality control, especially in the case of medicines, since this is an essential stage in good manufacturing practices, with regard to sampling, specifications, tests, together with

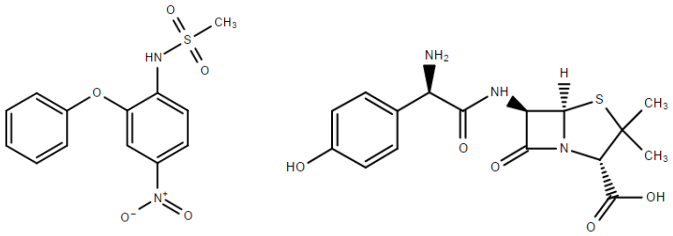
[**] Full text is published in JAS V. 85, No. 6 (http://springer.com/10812) and in electronic version of ZhPS V. 85, No. 6 (http://www.elibrary.ru/title_about.asp?id=7318; sales@elibrary.ru).

organizational and documentary procedures. These practices are designed to ensure that medical products are not offered for sale or used until their quality is considered to be satisfactory and reliable [4].The quality control of medicines has been the source of great anxiety owing to problems they can cause to the organism. This has led to a large number of research studies and made it essential to employ effective and reliable analytical methods [5].

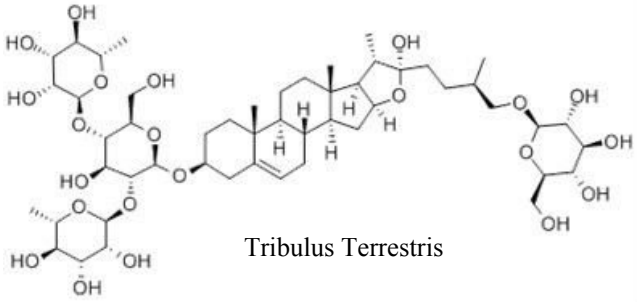
Nimesulide, which has an N-(4-nitro-2-phenoxyphenyl) methanesulfonamide molecular formula, is a nonsteroidal anti-inflammatory drug with anti-inflammatory, analgesic, and antipyretic action. It is widely prescribed and recommended since it is more effective than similar drugs, such as ibuprofen, diclofenac, and piroxicam [6]. However, this product leads to contradictory results in several cases, for example, in patients with hypersensitivity reactions (bronchospasm, rhinitis, hives, and gastrointestinal bleeding) [7]. Nimesulide has a history of side effects, such as gastric discomfort or pain, loss of appetite, nausea, vomiting, gastric bleeding, and a disorderly collection of some types of liver lesions [8].

Amoxicillin is a semisynthetic penicillin. As it forms a part of the amino group, it has a wide-ranging spectrum of action. In comparison with ampicillin, it has greater bioavailability, and the presence of food does not interfere with its absorption [9]. It is a resistant acid but undergoes inactivation of the beta-lactamases produced by several bacteria [10]. The maximum plasma concentrations of amoxicillin are reached in about two hours, with an average of 4 μg/ml [11]. It can be used for the treatment of respiratory tract infections, such as bronchitis, bacterial pharyngitis, acute otitis, sinusitis, and typhoid fever [12]. In addition to different infections caused by burns of the skin and soft tissue, the biliary and genitourinary tract can be treated with amoxicillin. Thus, it is necessary to establish surveillance mechanisms on the use of antibiotics [13].

Tribulus Terrestris forms a part of the composition of several drugs, and owing to its great importance in the treatment of various hormonal diseases, there has been a great deal of research on its control and monitoring. However, there have been few analytical methods for Tribulus Terrestris in clinical and pharmaceutical samples [14]. In view of its importance, there is a need to employ selective methods that are precise and fast, as well as cheap, for its detection in clinical and pharmaceutical samples. There is a need to detect the drug in several samples so that this metabolite can be monitored, as it is involved in several types of treatment [15].



Nimesulide Amoxicillin



Tribulus Terrestris

Other techniques used for the determination of drugs found in the literature are flow injectionanalysis (FIA) [16], chromatography [17], fluorimetry [18], and electrochemistry [19]. However, some of these procedures are expensive, while others are complicated and/or require well-trained professionals, since many of these procedures involve several analytical stages.

In light of this, the purpose of this work is to carry out the spectrophotometric determination of drugs in commercial and clinical samples for analytical control, with the aim of validating the method.

Experimental. *Reagents and equipment*. The reagents used were of analytical purity and included Tribulus Terrestris (Herbarium), nimesulide (Sigma), amoxicillin (Sigma), ethanol (JTBaker), isopropyl al-

cohol (JTBaker), tetrahydrofuran (Synth), acetone (Synth), and sodium hydroxide (Merck). The spectroscopy measurements were calculated by a Femto spectrophotometer, model 600 s with a 1.0 cm optical path quartz cell, connected to a Cole Parmer recorder (Niles, IL, USA), and gave absorbance readings between 300 and 700 nm. The Origin program from Microcal Inc. 7.0 © was used to handle the results obtained.

Preparation of the standards and construction of the analytical curve. A stock solution of 0.5 g/L of nimesulide was prepared in 100 mL of sodium hydroxide (NaOH) 0.1 mol/L. Seven stock solutions of nimesulide were prepared from the stock solution by dilution in the concentration ranges of 0.42 to 5.13 mg/L.

Amoxicillin was prepared in 1.0 mol/L NaOH in the ratio of 2:8 with ethanol/NaOH. Subsequently, a stock solution of amoxicillin of 0.5 g/L in 100 mL of NaOH was prepared. The stock solution allowed seven drug solutions to be prepared by dilution in the concentration ranges of 0.36 to 4.85 mg/L.

The Tribulus Terrestris was prepared in 1.0 mol/L NaOH. Subsequently a stock solution of Tribulus Terrestris of 0.2 g/L in 100 ml of NaOH was prepared. Seven stock solutions of the drug were prepared from the stock solution by dilution in concentration ranges from 0.18 to 1.00 mg/L.

A calibration curve was constructed with stock solutions prepared in glass cuvettes with a 1.0 cm optical path length and $\lambda = 460$ nm for nimesulide, $\lambda = 530$ nm for amoxicillin, and $\lambda = 350$ nm for Tribulus Terrestris. Each point on the calibration curve was repeated seven times. Statistical tests of linearity and homoscedasticity of variance were performed.

Preparation of clinical and commercial samples. The representative spectra ranged from 300–700 nm in the standard solution of 7.0 mg/L of nimesulide in 0.1 mol/L NaOH, 1.0 mg/L standard solution of Tribulus Terristris in 1.0 mol/L NaOH, and in the standard solution of 7.0 mg/L of amoxicillin in 2:8 ethanol/1.0 mol/L NaOH solution. Aliquots of 2 mL of these solutions were transferred to a glass pan of a 1.0 cm optical path.

Cimelide® (CIMED), containing nimesulide in its formula, Androsten® (Herbarium), containing Tribulus Terristris in its formula, and EMS®, GERMED®, Medley,® and Legrand®, containing amoxicillin in its formula, were obtained from local drugstores. All the commercial samples were prepared by crushing the tablets and diluting the substance to 100 mL of the stock solution. An aliquot of 50 μL was withdrawn from this solution and diluted to 10 mL of the stock solution, and then the spectrophotometric analysis was conducted. The analysis consists of three distinct solutions: (a) 2.0 mL of stock solutions; (b) 0.5 mL of the sample, plus 1.5 mL of the stock solution, and (c) 0.5 mL of the standard solution, plus 1.5 mL of the stock solution.

Clinical samples were performed in compliance with the standard method recommended by the Brazilian Pharmacopoeia [20]. First, blood and urine samples were collected from patients, 5.0 mL of blood and 50 mL of urine. Analyses of the drugs were performed by directly using the blood and urine of the patients, and the samples were recovered to analyze the efficiency of the proposed method. The uncertainties of the effectiveness of this method were assessed and compared with the target uncertainty and compatibility test devised by the Eurachem Guide/CITAC [21].

Results and discussion. *Absorption spectra and standardization of the methodology*. The solvent and the pH were first standardized in a suitable way for the detection of the drugs. Several proportions of ethanol and water were investigated for amoxicillin as well as the concentration of NaOH for nimesulide and Tribulus Terrestris so that the drug detection of the pharmaceutical samples could be optimized. Among the proportions studied, the ratio 2:8 ethanol/NaOH was chosen because it showed a better spectrophotometric response, and the NaOH concentration of 0.1 mol/L for nimesulide and 1.0 mol/L for amoxicillin and Tribulus Terrestris were also chosen.

Several solvents were tested, such as ethanol, isopropyl alcohol, tetrahydrofuran, acetone, and water. Among the solvents studied, water was chosen, because it provided a better spectrophotometric response for nimesulide and Tribulus Terrestris and ethanol/water for amoxicillin. The spectrophotometric experiments were carried out in alkaline media, since several substances do not have absorption peaks in an acidic or neutral medium, such as the case of the molecules of nimesulide and amoxicillin, but in an alkaline medium there are characteristic absorption peaks for their quantification [22]. Tribulus Terrestris was used in a basic medium because its dissolution does not occur in an acid or neutral medium. In the case of amoxicillin, an ethanol/water mixture was used because of the low solubility of the drug in an aqueous medium [23].

After the stock solutions had been optimized, representative spectra were performed for the drugs studied, as shown in Fig. 1. The maximum wavelengths obtained were as follows: 460 nm for the nimesulide solution, 530 nm for amoxicillin, and 350 nm for the Tribulus Terrestris solution; these wavelengths were used for the later experiments.

The molar absorptivity coefficient (ε) for the quantification of drug concentration by spectrophotometry was determined by means of the Lambert–Beer law [24]. Table 1 shows the molar absorptivity coefficient values for each of the analyzed drugs.

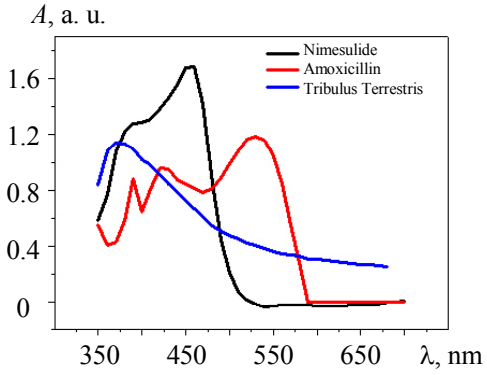


Fig. 1. Representative spectrum for nimesulide containing 7.0 mg/L of nimesulide in 0.1 mol/L NaOH, amoxicillin containing 7.0 mg/L of amoxicillin in 2:8 ethanol/1.0 mol/L NaO Hand, and Tribulus Terrestris containing 1.0 mg/L of Tribulus Terristris in NaOH 1.0 mol/L. The data correspond to a representative assay of seven independent experiments in 300–700 nm.

Owing to the slow reaction rate for amoxicillin in an alkaline medium, it was necessary to perform a kinetic study by varying the concentrations of NaOH and ethanol. The kinetic equation was first order for amoxicillin and zero order for NaOH ($v = k$[amoxicillin]) [25]. From the referent case study, it was observed that it is necessary to wait for a period of 30 min for the reaction to stabilize and subsequently carry out the experiments.

The calibration curve. After the working conditions for the drugs had been optimized, spectrophotometric measurements were made to obtain the calibration curve in an alkaline medium. Seven analytical curves were formed with different results to obtain repeatability data for the spectrophotometric measurements. This involved seven analytical curves based on seven different methods to obtain the curve reproducibility data [26]. Figure 2 shows the calibration curve for the spectrophotometric measurements of the analyzed drug Tribulus Terrestris. The other drugs behaved in a similar way, as shown in Table 1, which outlines the statistical parameters for the intercept and slope, as well as Pearson's linear correlation coefficient, the linear range and limits of detection and quantification obtained by the calibration curve. The results were satisfactory, with low limits of detection and quantification, as well as a good linear correlation in the calibration curve.

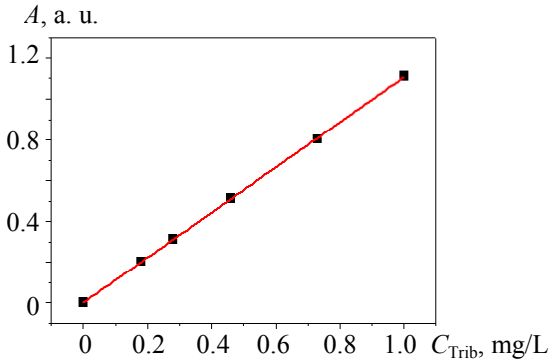


Fig. 2. Analytical curve obtained for detection of Tribulus Terrestris in 1.0 mol/L of NaOH with λ = 350 nm containing 0.18 to 1.0 mg/L of Tribulus Terrestris.

TABLE 1. Regression, Correlation, Homoscedasticity, Linearity, Molar Absorptivity, Limit of Detection, and Quantification and Concentration Range of Test Parameters for the Performed Standard Calibrations

Drug	*a*	*b*, L/mg	*r*	*n*	*F*	F_{LOF}	ε, L/(cm · g)	LOD, mg/L	LOQ, mg/L	Concentration range, mg/L
Nimesulide	0.0066	0.2377	0.9998	8	2.24	3.29	237.7	0.092	0.31	0.42–5.13
Amoxicillin	0.0006	0.1709	0.9995	8	3.86	2.19	170.9	0.14	0.48	0.36–4.85
Tribulus Terrestris	0.0036	1.111	0.9997	6	2.99	2.44	1111	0.019	0.066	0.18–1.0

N o t e. *a* and *b* are the intercept and slope of the regression line, *r* is Pearson's linear correlation coefficient, *F* and F_{LOF} are the calculated parameters of the Fisher and ANOVA-LOF tests corresponding to the critical values of 5.81 and 4.02 respectively, ε is molar absorptivity, LOD is limit of detection, and LOQ is limit of quantification.

The homogeneity of the instrumental response variances was evaluated by means of Fisher's tests [27], and the linearity through the ANOVA Lack-of-fit [28] test at a 95% confidence level.

Table 1 shows the linear regression and the correlation parameters estimated for the calibration curves and the parameters involved in the homoscedasticity and linearity tests of the signal. When the Fisher homoscedasticity test and Lack-of-fit ANOVA linearity are taken into account, all the calibrations have homoscedastic signals corresponding to the critical values of 5.81 and 4.02, respectively. This means that the least squares method can be applied to quantify the drugs by spectrophotometry in alkaline media.

In the case of nimesulide, the spectrophotometric response was linear and in the concentration range from 0 to 5.13 mg/L with a detection limit of 90 μg/L and a quantification limit of 0.3 mg/L, as expressed in the equation Abs = 0.0066 + 237.7[nimesulide/mg/L] (n = 8; R = 0.9998) [29]. With regard to amoxicillin, the spectrophotometric response was linear and in the concentration range from 0 to 4.85 mg/L with a detection limit of 0.14 mg/L and a quantification limit of 0.48 mg/L, as given in the equation Abs = 0.0006 + + 0.1709[amoxicillin/mg/L] (n = 8; R = 0.9995). In the case of Tribulus Terrestris, the spectrophotometric response was linear and in the concentration range from 0 to 1.00 mg/L with a detection limit of 19 μg/L and a quantification limit of 66 μg/L, in accordance with the equation Abs = 0.0036 + 1.111[Tribulus Terrestris/mg/L] (n = 6; R = 0.9998).

Application in real samples. The applicability of the developed spectrophotometric method was assessed by determining the contents of the drugs in clinical samples of blood and urine and commercial samples with tablets containing the medicine. The blood samples studied were from patients, and the blood was extracted following the procedure outlined in the materials and methods section.

The results obtained by the spectrophotometric method are shown in Table 2, which compares the value detected by the recovery in the clinical samples with the values tabulated in the tablets. All the results obtained have a number of associated uncertainties [30]. The recovery values were 1 to 7%. The results were less satisfactory in clinical specimens due to interferences in the blood and urine, but all the results passed the compatibility tests described earlier.

As observed in the results given in Table 2, the recommended spectrophotometric method was efficient in quantifying the drugs in the studied samples, where the values are close to the recovered value and those labeled as commercial samples. A preliminary statistical study was undertaken to observe the reproducibility of the method for drug detection by calculating the uncertainty parameters for each of the samples, as shown in Table 2.

All the results were shown with their respective uncertainties associated and combined with the law of propagation of uncertainties [31]. The expanded uncertainty (U) was calculated at a 95% confidence interval by multiplying the combined uncertainty by a factor of k = 2, as shown in Table 2. The sources of uncertainty were the stock solution (μ_s), sample volume (μ_v), dilution factor (μ_d), and extrapolation of the calibration curve (μ_e). All these uncertainties were calculated by adopting a bottom-up approach and combined in accordance with the equation [32]

$$\mu_\gamma = \gamma\sqrt{\mu_s^2 + \mu_v^2 + \mu_d^2 + \mu_e^2}, \quad (1)$$

where μ_γ is the uncertainty of its experimental value and γ is the reference value.

The calculated expanded uncertainty was compared with the target uncertainty in accordance with the EURACHEM/CITAC[21] guidelines and followed the criterion of being up to 10% of the experimental value. Table 2 shows the percentage of uncertainties for each of the samples, and all of them had a maximum of 10%, which is in line with the adopted criteria for target uncertainty.

TABLE 2. Results of the Spectrophotometric Determinations of the Studied Drugs. These Show the Absorption of Each Sample, Estimated Reference Concentration with Regard to its Respective Uncertainty, Compatibility Test, and Target Uncertainty

Drug	Sample	Absorption	Estimated concentration γ_E, spectroscopy method	Reference concentration γ_R, mg/L ***	Compatibility test (absolute difference, $\lvert\gamma_E - \gamma_{Ref}\rvert$), mg/L	Target uncertainty, %
Nimesulide	Blood A	0.306	5.05±0.23*	5.00±0.05	0.05±0.47	4.6
	Blood B	0.311	5.12±0.35*	5.00±0.05	0.12±0.70	6.8
	Urine A	0.319	5.25±0.44*	5.00±0.02	0.25±0.88	8.4
	Cimelide©	0.326	107±4.35**	100±1.2	7.00±8.98	4.1
Amoxicillin	Blood C	0.207	4.84±0.13*	5.00±0.05	0.16±0.28	2.7
	Urine B	0.226	5.29±0.29*	5.00±0.02	0.29±0.58	5.5
	EMS©	0.219	512±13.7**	500±6.2	12.0±29.9	2.7
	GERMED©	0.217	508±15.4**	500±6.2	8.00±33.0	3.0
	MEDLEY©	0.216	505±14.3**	500±6.2	5.00±31.0	2.8
	LEGRAND©	0.222	869±32.1**	875±9.4	6.00±66.6	3.7
Tribulus Terrestris	Blood D	0.596	2.13±0.17*	2.00±0.03	0.13±0.34	8.0
	Urine C	0.697	2.48±0.25*	2.00±0.01	0.48±0.50	10.1
	Androsten©	0.494	89±3.26**	94±0.09	5.00±6.49	3.7

The increased degree of uncertainty was estimated for a confidence value of 95%.
* Samples of blood and urine in units of mg/L.
** Commercial sample measurement units in mg/tablet.
*** The comparative reference values were the recovery and tabulated values in the packages with their associated degrees of uncertainty.

A compatibility test was conducted to evaluate the reliability of the proposed method by comparing the experimental value with the reference value and taking account of the uncertainties of the experimental values and the reference values, as shown in the equation [33]:

$$\lvert \gamma_E - \gamma_R \rvert \le t_E^{99\%} \sqrt{u^2(\gamma_E) + u^2(\gamma_R)}, \quad (2)$$

where γ_E and γ_R are the experimental and reference values, respectively; $u^2(\gamma_E)$ is the uncertainty associated with the experimental and reference values; and $t_E^{99\%}$ is the t-value of student for the number of experimental repetitions for 99% confidence and relative degrees of freedom u_E.

If this condition is true, the proposed method is compatible with the adopted reference values [33]. The uncertainty of the standards was calculated by estimating the uncertainties of the preparation and recovery of the samples. The uncertainties that took into account the reference value were the uncertainty of the stock solution (μ_s), sample volume (μ_v), and dilution factor (μ_d).

On the basis of the data obtained in Table 2, the spectrophotometric method in an alkaline medium behaved in a satisfactory and acceptable way for the spectrophotometric technique of quantification and meant that the application of the method employed in the quantification of the medicines in clinical and commercial samples was feasible. The values with their respective uncertainties are compatible with the target uncertainties.

Conclusion. The use of the spectrophotometric method in an alkaline medium proved to be very efficient in preparing a new spectrophotometric method for the determination of nimesulide, amoxicillin, and Tribulus Terrestris in clinical and commercial samples. The experimental results show that the method developed is promising for determination of these drugs in different types of real samples. The absorbance obtained was proportional to the concentration of the drugs, and the method had low detection limits during the spectrophotometric measurements. In view of its sensitivity, stability, operational simplicity, and cost-effectiveness, the method developed is highly efficient in the determination of these medications.

Acknowledgment. The students would like to express their thanks to K.F.D.P., L.N.O., and M.J.S. and the Fundação Educacional de Fernandópolis for creating a suitable framework and offering an incentive for this academic undertaking.

REFERENCES

1. H. N. Deepakumari, M. K. Prashanth, B. C. Vasantha Kumar, H. D. Revanasiddappa, *J. Appl. Spectrosc.*, **81**, 1004–1011 (2015).
2. N. Rajendraprasad, K. Basavaiah, *J. Appl. Spectrosc.*, **83**, 392–397 (2016).
3. M. M. Moein, A. El Beqqali, M. Abdel-Rehim, *J. Chromatogr. B*, **1043**, 3–11 (2017).
4. T. T. He, C. O. L. Ung, H. Hu, Y. Wang, *Eur. J. Integr Med.*, **7**, 55–66 (2015).
5. P. Kaur, S. K. Singh, M. Gulati, Y. Vaidya, *J. Appl. Spectrosc.*, **82**, 1060–1063 (2016).
6. X. Sun, K. Xue, X. Jiao, Q. Chen, L. Xu, H. Zheng, Y. Ding, *J. Chromatogr. B*, **1027**, 139–148 (2016).
7. A. P. Dewani, S. M. Dabhade, R. L. Bakal, C. K. Gadewar, A. V. Chandewar, S. Patra, *Arab. J. Chem.*, **8**, 591–598 (2015).
8. I. M. P. Silva, M. A. Carvalho, C. S. Oliveira, D. M. Profirio, R. B. Ferreira, P. P. Corbi, *Inorg. Chem. Commun.*, **70**, 47–50 (2016).
9. L. M. M. Lê, L. Eveleigh, I. Hasnaoui, P. Prognon, A. Baillet-Guffroy, E. Caudron, *J. Pharm. Biomed. Anal.*, **138**, 249–255 (2017).
10. K. Shah, E. Hassan, F. Ahmed, I. Anis, M. Rabnawazand, R. Shah, *Ecotoxicol. Environ. Saf.*, **141**, 25–29 (2017).
11. M. Ahmadi, T. Madrakian, A. Afkhami, *Talanta*, **148**, 122–128 (2016).
12. M. N. Uddin, S. Das, S. H. Khan, S. K. Shill, H. R. Bhuiyan, R. Karim, *J. Taibah Univ. Sci.*, **10**, 755–765 (2016).
13. L. Shen, H. Wang, P. Chen, C. Yu, Y. Liang, C. Zhang, *J. Food Drug. Anal.*, **24**, 199–205 (2016).
14. M. Ganzera, E. Bedir, I. A. Khan, *J. Pharm. Sci.*, **90**, 1752–1758 (2001).
15. A. Saiyed, N. Jahan, S. Ajij A. Makbul, M. Ansari, H. Bano, S. H. Habib, *Integ. Med. Res.*, **5**, 293–300 (2016).
16. B. C. Lourencao, R. A. Medeiros, O. Fatibello-Filho, *J. Electroanal. Chem.*, **754**, 154–159 (2015).
17. A. Petruczynik, K. Wróblewski, M. Szultka-Młyńska, B. Buszewski, H. Karakuła-Juchnowicz, J. Gajewski, J. Morylowska-Topolska, M. Waksmundzka-Hajnos, *J. Pharm. Biomed. Anal.*, **127**, 68–80 (2016).
18. M. S. Chernov'yants, A. O. Dolinkin, A. V. Chernyshev, E. V. Khohlov, E. G. Golovanova, *J. Pharm. Sci.*, **99**, 1567–1573 (2010).
19. T. R. L. Dadamos, B. H. Freitas, D. H. M. Gênova, R. D. Espírito-Santo, E. R. P. González, S. Lanfredi, Marcos F. S. Teixeira, *Sensors Actuators B: Chem.*, **169**, 267–273 (2012).
20. *ANVISA. Agencia Nacional De Vigilância Sanitária. Farmacopeia Brasileira*, Vol. 1, 5th ed., Brasilia (2010b).
21. S. L. R Ellison, A. Williams, *Eurachem/CITAC guide: Quantifying Uncertainty in Analytical Measurement*, 3rd ed., London, Eurachem (2012).
22. A. A. Gouda, M. I. K. El-Sayed, A. S. Amin, R. E. Sheikh, *Arab. J. Chem.*, **6**, 145–163 (2013).
23. V. J. Pereira, R. L. Matos, S. G. Cardoso, R. O. Soares, G. L. Santana, G. M. N. Costa, S. A. B. Vieira de Melo. *J. Supercrit. Fluids*, **81**, 128–146 (2013).
24. W. Mäntele, E. Deniz, *Spectrochim. Acta, A*, **173**, 965–968 (2017).
25. P. Atkins, *Físico-Química: Fundamentos*, 3rd ed., LTC (2003).
26. D. Djekic, R. Pinto, P. A. Vorkas, *Int. J. Cardiol.*, **222**, 1042–1048 (2016).
27. S. L. R. Ellison, T. J. Farrant, V. Barwick, *Practical Statistics for the Analytical Scientist: A Bench Guide*, Royal Society of Chemistry, UK (2009).
28. R. J. N. B. Silva, *Talanta*, **148**, 177–190 (2016).
29. T. R. L. Dadamos, M. F. S. Teixeira, *Electrochim. Acta*, **54**, 4552–4558 (2009).
30. I. Kuselman, F. Pennecchi, R. J. N. B. Silva, D. B. Hibbert, *Talanta*, **164**, 189–195 (2017).
31. M. Sega, F. Pennecchi, S. Rinaldi, F. Rolle, *Anal. Chim. Acta*, **920**, 10–17 (2016).
32. E. Theodorsson, *Clin. Lab. Med.*, **37**, 15–34 (2017).
33. *Joint Committee for Guides in Metrology, International Vocabulary of Metrology-Basic and General Concepts and Associated Terms (VIM)*, JCGM 200, BIPM (2012).